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# Exploring marine-derived fungal preussin toxicity on MDA-MB-231 cells cultured in 2D and 3D

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## Introduction & aim

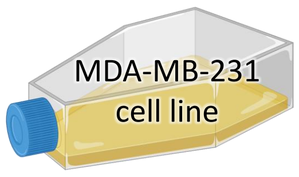
Triple-negative breast cancer (TNBC) has a poor prognosis, and limited therapeutic options[1], which allied with chemotherapy-associated toxicity and drug resistance call for the search for new molecules that may serve as new drugs, drug adjuvants, or scaffolds for drug development.

Preussin (Pr) is a natural compound shown to decline cell viability and proliferation and induce cell death and cell cycle arrest, mainly in bidimensional (2D) cell cultures of various cell lines [2–4].

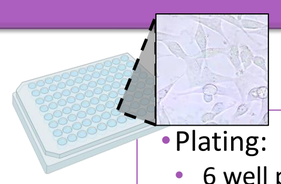
Three-dimensional (3D) cell cultures are described to better mimic the tumor behaviour *in vivo*[5].

This work aimed to explore better the effects of Pr on cell viability, proliferation, death induction, genotoxicity, and migration of a TNBC cell line, comparing its effects in 2D and 3D cell cultures.

## Method

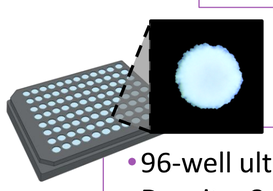



- DMEM high glucose, without glutamine and phenol red
- 1% penicillin/ streptomycin
- 5% FBS
- Incubated at 37 °C, 5% $CO_2$



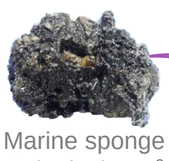
**2D cell culture**

- Plating:
  - 6 well plates – $2x10^5$ cells/well
  - 24 well plates – $5x10^4$ cells/well
  - 96 well plates – $5x10^3$ cells/well
- 24 h incubation for cell adhesion



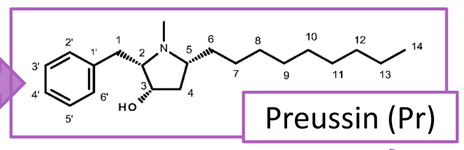
**3D cell culture**

- 96-well ultra-low attachment U-bottom plates
- Density: $8x10^4$ cells/well
- 72 h incubation for spheroid formation



Marine sponge *Epipolasis sp.*[2]

*Aspergillus candidus*




Freshly diluted in DMEM + 0.1% DMSO

| Assay | Output | Cell culture model | Incubation (h) | Concentration (µM) |
|---|---|---|---|---|
| MTT | Viability | 2D | 24, and 72 | 5, 15, 25, 35, and 50 |
| | | 3D | 96 | 25, 35, and 50 |
| BrdU | Proliferation | 2D | 72 (2D cell culture) | 25, and 35 |
| Annexin V-PI | Cytotoxicity | 3D | 96 (3D cell culture) | |
| TEM | Ultrastructure | 3D | 96 (3D cell culture) | |
| Comet assay • Alkaline • FPG | Genotoxicity | 2D and 3D | 2, and 24 | |
| Wound healing | Migration | 2D | 24, 48, and 72 | 5, 15, and 25 |

### Statistical analysis

- n = 5 independent experiments
- Normality and homogeneity of variance : Shapiro-Wilk's and Levene's tests
- Differences from the control group (C): One-way ANOVA followed by Holm-Šídák test
- Data are presented as mean and standard deviation. Significant differences are shown with asterisks (* $p < 0.05$; ** $p < 0.01$; $p < 0.001$; **** $p < 0.0001$)

## Acknowledgments

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## References

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## Results & discussion

**Fig. 1.A**

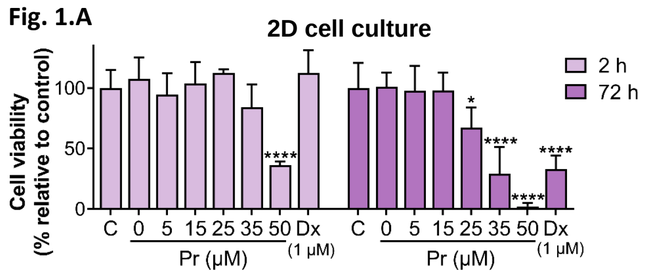



**Fig. 1.B**

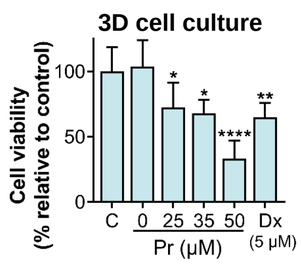



**Fig. 1.** Cell viability of MDA-MB-231 cell line after 24 and 72 h (2D cultures) (**A**) or 96 h (3D cultures) (**B**) of exposure to Pr, C or positive control of doxorrubicin (Dx).

**Fig. 2**

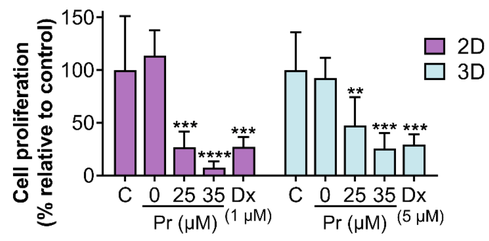



**Fig. 2.** Cell proliferation of MDA-MB-231 cell line after 72 h (2D cultures) or 96 h (3D cultures) of exposure to Pr, C or Dx.

**Fig. 3**

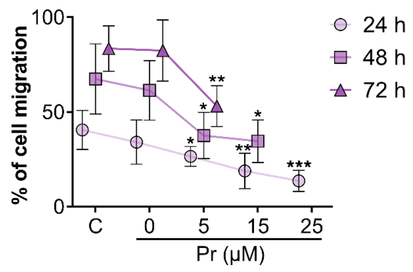



**Fig. 3.** Cell migration of MDA-MB-231 cell line after 24, 48 and 72 h of exposure to Pr, or C (DMEM with 0.5% FBS).

**Fig. 4. A**

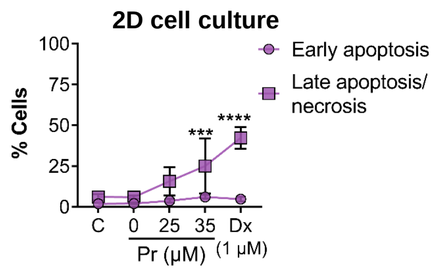



**Fig. 4. B**

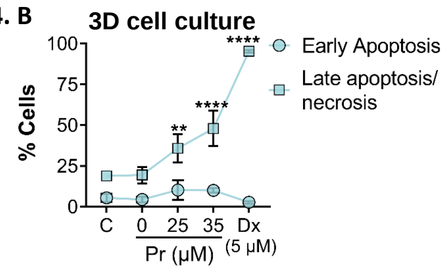



**Fig. 4.** Cell death (late apoptosis/necrosis) and early apoptosis of MDA-MB-231 cell line after 72 h (2D cultures) (**A**) or 96 h (3D cultures) (**B**) of exposure to Pr, C or Dx.

**Fig. 5.A.**

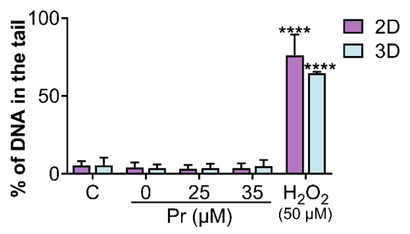



**Fig. 5.B.**

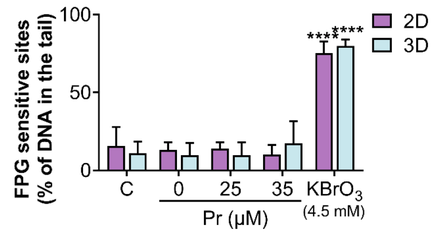



**Fig. 5.** DNA damage (strand breaks (**A**) and FPG sensitive sites (**B**)) of MDA-MB-231 cell line after 24 h of exposure to Pr, C or respective positive control.

With 2 h of exposure, similar results were obtained (data not shown).

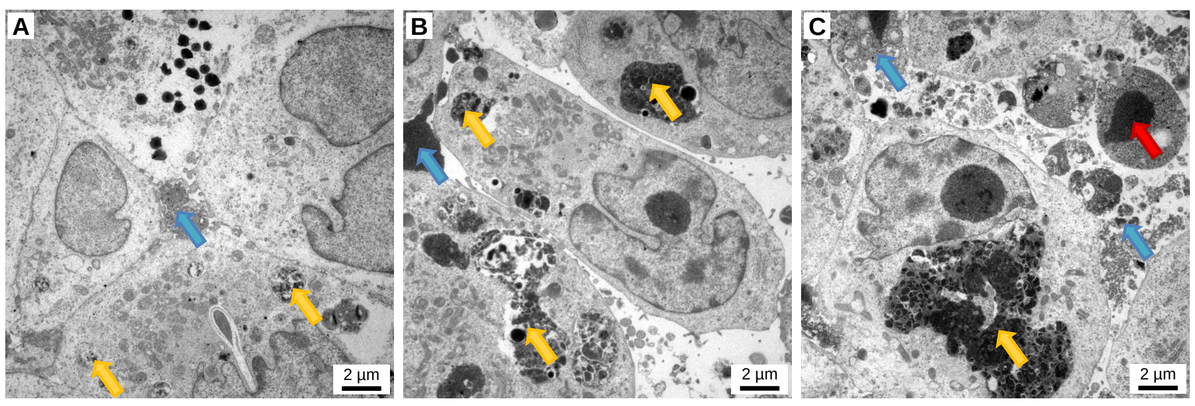
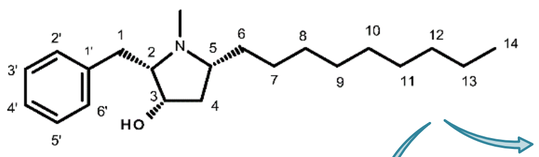



**Fig. 6.** Representative images of TEM of MDA-MB-231 cell line cultured in 3D culture models after exposure to C (**A**) or, 25 (**B**) or, 35 µM (**C**) of Pr. Blue arrow: cell debris; Red arrow: cells with morphology compatible with apoptosis; Yellow arrow: multivesicular dense bodies.

With the increase in concentrations of Pr, there was a rise in cell debris, intracellular multivesicular dense bodies, and cells with morphology compatible with apoptosis in both culture models (data from the 2D culture was not shown).

## Conclusion




- Cell viability ↓
- Cell proliferation ↓
- Cytotoxicity ↑ (Late-apoptosis and necrosis)
- Genotoxicity ✕ (Strand breaks, alkali-labile sites and FPG sensitive sites)

**Preussin has relevant proprieties that can be further explored**

Similar dose-dependent response of the MDA-MB-231 cell line in 2D and 3D cultures